Synthesis of new bioisosteric hemiasterlin analogues with extremely high cytotoxicity

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Synthesis of New Simplified Hemiasterlin Derivatives with *α,β*-Unsaturated Carbonyl Moiety

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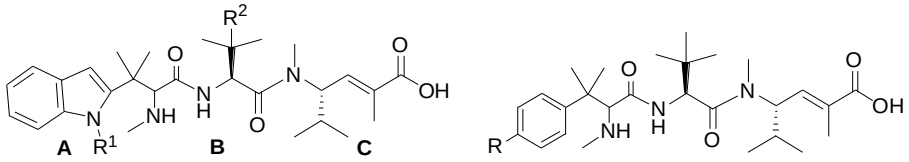
ABSTRACT
In this article, we report a convenient and efficient method for the synthesis of new simplified derivatives of hemiasterlin in which the *α,α*-dimethylbenzylic moiety A is replaced by *α,β*-unsaturated aryl groups as Michael acceptor. Most of these derivatives have a strong cytotoxic activity on three human tumor cell lines (KB, Hep-G_2 and MCF_7). Analogs **17b** and **17f** showed a high cytotoxicity against KB and Hep-G_2 cancer cell lines comparable to paclitaxel and ellipticine.

Hemiasterlins belong to a family of naturally occurring tripeptides from marine sponges.[1] The important derivatives of hemiasterlins are hemiasterlin A, hemiasterlin B, and hemiasterlin C, which were isolated from marine sponge *Auletta* and *Cymbastella* (Fig. 1) and exhibited potent cytotoxicity in vitro against murine leukemia P388 and human breast, ovarian, colon, and lung cancer cell lines.[2,3] Hemiasterlins suppress microtubule depolymerization presumably by binding to the vinca-alkaloid binding-domain of tubulin and leading to mitotic arrest and cell death.[4a] The synthetic analog HTI-286 (**2**) displayed especially potent cytotoxicity against paclitaxel (Taxol™) resistant turmor cell lines in vitro and in vivo and is currently in clinical trials.[4b]



Hemiasterlin (1) R^1 = Me R^2 = Me
Hemiasterlin A R^1 = H R^2 = Me
Hemiasterlin B R^1 = H R^2 = H
Hemiasterlin C R^1 = Me R^2 = H

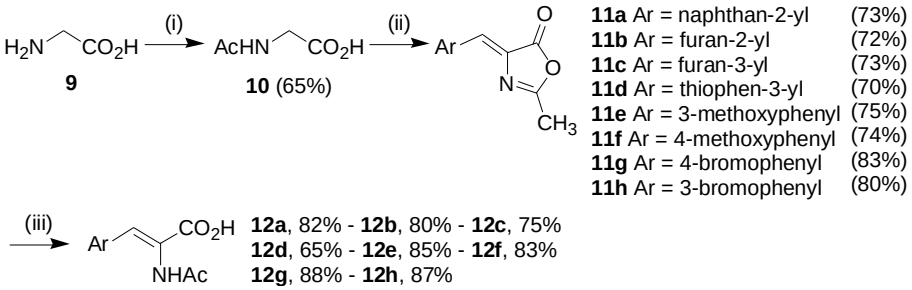
2 (HTI-286 R = H)
3 (HTI-286 analog R = OMe)

Figure 1. Structures of hemiasterlin derivatives and HTI analogs.

There are several reports on the synthesis of new derivatives of hemiasterlin in which the indole aromatic ring in the moiety **A** of the original molecule was replaced by aryl functional groups.[5,6a] However, asymmetric synthesis of the stereospecific amine group and especially the *gem*-dimethyl moiety were proved to be highly problematic. To overcome this difficulty, several studies explored modifications of segment **A** in which the *gem*-dimethyl moiety has been eliminated. Some of these derivatives showed promising cytotoxic activity.[6]

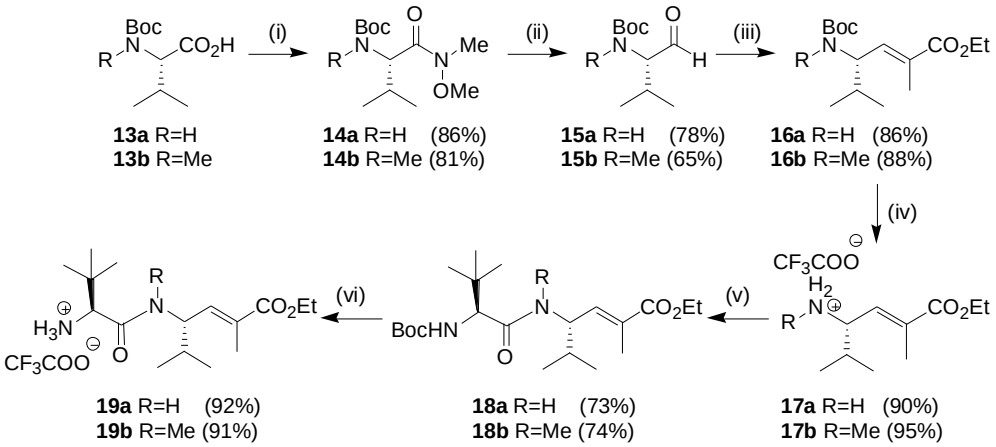
In recent years, previous works have shown that the presence of a *α,β*-unsaturated carbonyl system in peptide derivatives can improve the biological activity compared to the initial compound.[7,8] Explanations may include either a limitation of free energy of the peptide by addition of a constrained system or by an electrophilic character of the compound leading to the possibility to form covalent bond with the protein target. Based on the idea that hemiasterlin derivatives containing *α,β*-unsaturated carbonyl systems could induce a remarkable cytotoxicity, we decided to synthesize new hemiasterlin derivatives in which the *α,α*-dimethylbenzylic group (fragment A) is replaced by a *α,β*-unsaturated carbonyl group.

New hemiasterlin derivatives were synthesized via classical peptide coupling reactions between two fragments **7a-h** and **14a,b**. A general procedure for the synthesis of compound **7** is outlined in Scheme 1. Compounds **7** were prepared from glycine in three steps.[9] The synthesis started by acetylation of glycine with acetic anhydride in water at room temperature following by condensation with aryl aldehydes using sodium acetate in the presence of acetic anhydride at 90 °C for 12 h which afforded azalactones **6a-h** in 72-83% yields.[9] Finally, azalactones **6a-h** were hydrolyzed in aqueous sodium hydroxide, followed by treatment with hydrochloric acid (12 N) at 100 °C for 4 h to give compounds **7a-h** in 65-88% yields.



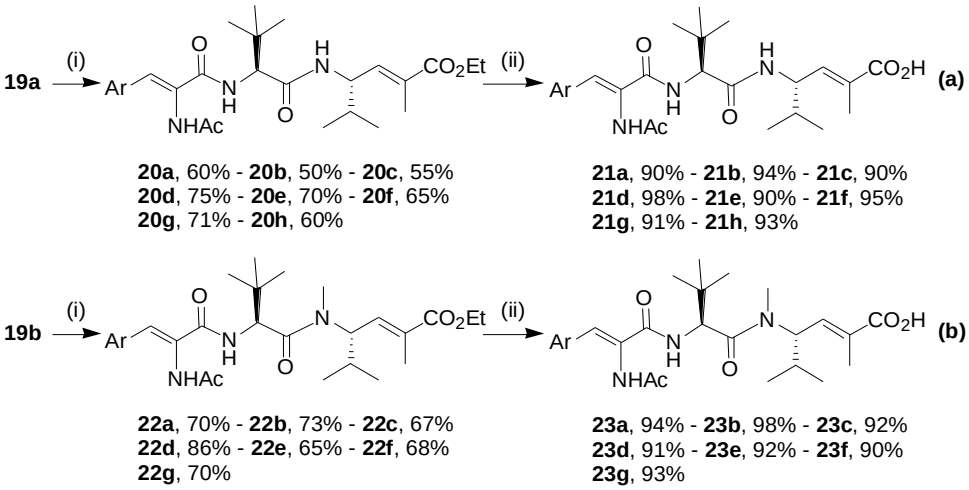
Scheme 1. *Reagents and conditions* (i) 2.0 equiv Ac_2O, H_2O, rt, 20 h. (ii) 0.75 equiv ArCHO, 1.0 equiv AcONa, Ac_2O, 90 °C, 12 h. (iii) NaOH (1 N) then HCl (12 N), 100 °C, 4 h.

Compounds **14a,b** were obtained from Boc-L-valine in 6 steps as depicted in Scheme 2.[4] In the first step, Boc-L-valines **8a,b** were converted to Weinreb amide **9a,b** in good yields (86 and 81%) by treatment with a mixture of *N,O*-dimethylhydroxylamine hydrochloride, 1-ethyl-3-(3-dimethylaminoproryl)carbodiimide (EDC) and hydroxybenzotriazole (HOBt) in the presence of N-ethylisopropylamine (*i*-PrNHEt) at room temperature for 12 h.[10] Reduction of **9a,b** using $LiAlH_4$ in THF was carried out at room temperature for 1 h to give aldehydes **10a,b** in 78% and 65% yields, respectively.[11] Afterward, Wittig reaction of aldehydes **10a,b** with ethyl 2-(triphenylphosphoranylidene)propanoate was carried out at reflux in CH_2Cl_2 for 6 h to afford the alkenoates **11a** and **11b** in 86% and 88% yields, respectively.[6,12] Then, removal of the Boc group using trifluoroacetic acid in CH_2Cl_2 at room temperature for 1 h led to **12a,b** in high yields (90 and 95%). The expected compounds **14a,b** are finally obtained after coupling **12a,b** and Boc-L-leucine in the presence of EDC/HOBt in DMF at room temperature for 12 h following by Boc deprotection in classical reaction conditions.



Scheme 2. *Reagents and conditions* (i) 1.1 equiv NH(Me)OMe, 1.1 equiv EDC, 1.1 equiv HOBt, 2.0 equiv *i*-PrNHEt, DMF, rt, 12 h. (ii) 4.0 equiv LAH, THF, rt, 1 h. (iii) $Ph_3P{=}C(CH_3)CO_2Et$, CH_2Cl_2, reflux, 6 h. (iv) TFA, CH_2Cl_2, rt, 1 h. (v) 1.0 equiv Boc-L-Leucine, 1.1 equiv EDC, 1.1 equiv HOBt, 2.0 equiv *i*-PrNHEt, DMF, rt, 12 h. (vi) TFA, CH_2Cl_2, rt, 1 h.

The final compounds **16a-h** were then prepared in two steps after peptide coupling reactions of **14a** with amides **7a-h** following by the saponification of ester using a 1 N lithium hydroxide solution (Scheme 3-a). Similarly, the hemiasterlin derivatives **18a-g** were obtained from **14b** (Scheme 3-b).



Scheme 3. *Reagents and conditions* (i) 1.0 equiv **12a-h**, 1.1 equiv EDC, 1.1 equiv HOBt, 2.0 equiv *i*-PrNHEt, DMF, rt, 12 h. (ii) 10 equiv LiOH, MeOH : H_2O (2:1), rt, 10 h.

All compounds **15a-h**, **16a-h**, **17a-g** and **18a-g** were evaluated in vitro for their cytotoxic activity against four human tumor cell lines (KB, Hep-G_2, LU and MCF_7) and the results were summarized in Table 1. Eleven hemiasterlin derivatives showed strong activity against the KB, Hep-G_2 cell line with IC_{50} value below 100 nM. Analogs **16b**, **17b** and **18f** exhibited potent cytotoxicity against the KB cell line with IC_{50} = 8.2, 3.5 and 3.7 nM, respectively. Meanwhile analogs **16b**, **2217c** and **17f** displayed potent cytotoxicity against the Hep-G_2 cell line with IC_{50} value 17.8, 16.3 and 3.7 nM, respectively. Derivatives **15a**, **15e** and **17a** presented a cytotoxic activity against MCF_7 cell line with IC_{50} value 42.3, 60.0 and 67.9 nM. Concerning the last cell line, the LU cell line, the hemiasterlin analogs showed weak activities with IC_{50} values above

269 nM. It is noteworthy to mention that two derivatives **17a** and **17f** present a cytotoxic activity against two cancer cell lines (KB, Hep-G_2) comparable with those of ellipticine and paclitaxel

Table 1. Cytotoxicity evaluation.

Entry	compound	IC_{50} (nM)			
		KB	Hep-G_2	LU	MCF_7
1	**15a**	**30.8**	**39.7**	>269	**42.3**
2	**15b**	160.5	214.2	>269	>269
3	**15c**	**86.2**	**67.3**	>269	>269
4	**15d**	107.4	**57.6**	>269	203
5	**15e**	**77.4**	**47.7**	228.4	**62**
6	**15f**	>269	>269	>269	>269
7	**15g**	>269	>269	>269	>269
8	**15h**	>269	>269	>269	>269
9	**6a**	**63**	**33.4**	>269	>269
10	**6b**	**8.2**	**17.8**	>269	>269
11	**6c**	155.4	>269	>269	>269
12	**6d**	>269	>269	>269	>269
13	**6e**	>269	>269	>269	>269
14	**6f**	>269	>269	>269	>269
15	**6g**	>269	>269	>269	>269
16	**6h**	>269	>269	>269	>269
17	**17a**	**23.0**	**23.1**	>269	**67.9**
18	**17b**	**3.5**	**31.4**	231.0	178.4
19	**17c**	**24.1**	**16.3**	158	112.8
20	**17d**	202	**15.0**	>269	>269
21	**17e**	>269	>269	>269	>269
22	**17f**	**3.7**	**3.7**	>269	215
23	**17g**	**49.0**	>269	>269	>269
24	**18a**	**69.7**	**13.0**	>269	>269
25	**18b**	188.0	>269	>269	>269
26	**18c**	>269	149.4	>269	>269
27	**18d**	234	**63.2**	>269	>269
28	**18e**	>269	>269	>269	>269
29	**18f**	**15.8**	**63.8**	>269	>269
30	**18g**	>269	>269	>269	>269
31	**Ellipticine**	**1.26**	**1.26**	**1.82**	**2.15**
32	**Paclitaxel**	**3.9**	**0.19**	**-**	**-**

In conclusion, a concise synthetic approach for new modified hemiasterlin derivatives was achieved in which the α,α-dimethylbenzylic group and amino NHMe moiety were replaced respectively by a α,β-unsaturated aryl and an amide NH-Ac group avoiding the synthesis of the chiral fragment **A**. Most of these derivatives possess strong cytotoxic activity on three human tumor cell lines (KB, Hep-G_2 and MCF_7) and two analogs, **17b** and **17f**, showed a comparable cytotoxicity activity to paclitaxel and ellipticine against KB and Hep-G_2 cancer cell lines. Based on previously reported work,[6b] we can envisage that our hemiasterlin derivatives act as tubulin

polymerization inhibitors. From our best compounds, more detailed biological studies will be undertaken and will be reported in due course.

Acknowledgments

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Supplementary Material

Supplementary data associated with this article can be found, in the online version, at doi:

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